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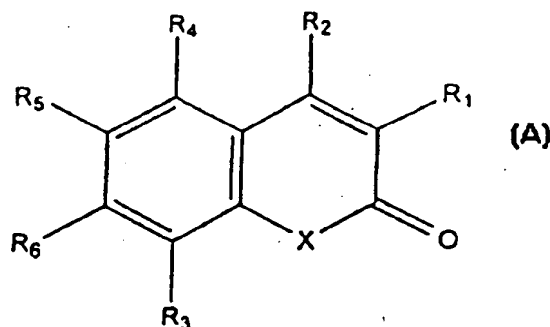
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(54) Title: NON-STEROIDAL POLYCYCLIC RING SULPHAMATE DERIVATIVES, THEIR PREPARATION AND THEIR USE AS OESTRONE SULPHATASE INHIBITORS

(57) Abstract

A compound is described. In particular, a non-steroidal sulphamate compound is described. The compound is suitable for use as an inhibitor of oestrone sulphatase. The compound has a polycyclic ring structure comprising two or more rings wherein at least two of the rings mimic the A and B rings of oestrone. Preferably the compound has general formula (A), wherein R₁-R₆ are independently selected from H, halo, hydroxy, sulphamate, alkyl and substituted variants or salts thereof; but wherein at least one of R₁-R₆ is a sulphamate group; and wherein X is any one of O, S, NH, a substituted N, CH₂, or a substituted C.



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NON-STEROIDAL POLYCYCLIC RING SULPHAMATE DERIVATIVES, THEIR PREPARATION AND THEIR USE AS OESTRONE SULPHATASE INHIBITORS

The present invention relates to a compound.

- 5 In particular the present invention relates to a non-steroidal compound and to a pharmaceutical composition comprising the non-steroidal compound.

Evidence suggests that oestrogens are the major mitogens involved in promoting the growth of tumours in endocrine-dependent tissues, such as the breast and
10 endometrium. Although plasma oestrogen concentrations are similar in women with or without breast cancer, breast tumour oestrone and oestradiol levels are significantly higher than in normal breast tissue or blood. *In situ* synthesis of oestrogen is thought to make an important contribution to the high levels of oestrogens in tumours and therefore specific inhibitors of oestrogen biosynthesis are of potential value for the
15 treatment of endocrine-dependent tumours.

Over the past two decades, there has been considerable interest in the development of inhibitors of the aromatase pathway which converts the androgen precursor androstenedione to oestrone. However, there is now evidence that the oestrone
20 sulphatase (E1-STS) pathway, i.e. the hydrolysis of oestrone sulphate to oestrone (E1S to E1), as opposed to the aromatase pathway, is the major source of oestrogen in breast tumours^{1,2}. This theory is supported by a modest reduction of plasma oestrogen concentration in postmenopausal women with breast cancer treated by aromatase inhibitors, such as aminoglutethimide and 4-hydroxyandrostenedione^{3,4} and
25 also by the fact that plasma E1S concentration in these aromatase inhibitor-treated patients remains relatively high. The long half-life of E1S in blood (10-12 h) compared with the unconjugated oestrogens (20 min)⁵ and high levels of steroid sulphatase activity in liver and, normal and malignant breast tissues, also lend support to this theory⁶.

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PCT/GB92/01587 teaches novel steroid sulphatase inhibitors and pharmaceutical compositions containing them for use in the treatment of oestrone dependent tumours, especially breast cancer. These steroid sulphatase inhibitors are sulphamate esters, such as N,N-dimethyl oestrone-3-sulphamate and, preferably, oestrone-3-sulphamate
5 (otherwise known as "EMATE").

EMATE is a potent E1-STS inhibitor as it displays more than 99% inhibition of E1-STS activity in intact MCF-7 cells at 0.1 μ M. EMATE also inhibits the E1-STS enzyme in a time- and concentration-dependent manner, indicating that it acts as an
10 active site-directed inactivator^{7,8}. Although EMATE was originally designed for the inhibition of E1-STS, it also inhibits dehydroepiandrosterone sulphatase (DHA-STS), which is an enzyme that is believed to have a pivotal role in regulating the biosynthesis of the oestrogenic steroid androstenediol^{8,9}. Also, there is now evidence to suggest that androstenediol may be of even greater importance as a promotor of
15 breast tumour growth¹⁰. EMATE is also active *in vivo* as almost complete inhibition of rat liver E1-STS (99%) and DHA-STS (99%) activities resulted when it is administered either orally or subcutaneously¹¹. In addition, EMATE has been shown to have a memory enhancing effect in rats¹⁴. Studies in mice have suggested an association between DHA-STS activity and the regulation of part of the immune
20 response. It is thought that this may also occur in humans^{15,16}. The bridging O-atom of the sulphamate moiety in EMATE is important for inhibitory activity. Thus, when the 3-O-atom is replaced by other heteroatoms (Figure 1) as in oestrone-3-N-sulphamate (4) and oestrone-3-S-sulphamate (5), these analogues are weaker non-time-dependent inactivators¹².

25

Although optimal potency for inhibition of E1-STS may have been attained in EMATE, it is possible that oestrone may be released during sulphatase inhibition^{8,12}, and that EMATE and its oestradiol congener may possess oestrogenic activity¹³.

30 The present invention therefore seeks to provide compounds suitable for the inhibition of E1-STS but which have no, or a minimal, oestrogenic effect.

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According to a first aspect of the present invention there is provided a non-steroidal sulphamate compound suitable for use as an inhibitor of oestrone sulphatase, wherein the compound has a polycyclic ring structure comprising at least a first ring and a second ring; wherein the first ring and the second ring mimic the A and B rings of oestrone; and wherein the polycyclic ring structure is not tetrahydro-naphthol.

Preferably either the first ring or the second ring comprises an α,β -unsaturated lactone group.

10 Preferably the first ring is a phenolic ring.

Preferably the compound has a bicyclic ring structure.

15 Preferably the compound has the general Formula (A) (see Figure 8). In Formula (A) R_1-R_6 are independently selected from H, halo, hydroxy, sulphamate, alkyl and substituted variants or salts thereof; but wherein at least one of R_1-R_6 is a sulphamate group; and wherein X is any one of O, S, NH, a substituted N, CH_2 , or a substituted C.

20 Preferably X is O.

Preferably, if the sulphamate group of the compound were to be replaced with a sulphate group to form a sulphate compound then that sulphate compound would be hydrolysable by an enzyme having steroid sulphatase (E.C. 3.1.6.2) activity.

25

In a highly preferred embodiment, the compound is not hydrolysable by an enzyme having steroid sulphatase (E.C. 3.1.6.2) activity.

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According to a second aspect of the present invention there is provided a non-steroidal compound wherein the compound has the general Formula (B) (see Figure 8). In Formula (B) R_1 - R_6 are independently selected from H, halo, hydroxy, sulphamate, alkyl and substituted variants or salts thereof; but wherein at least one of R_1 - R_6 is a sulphamate group.

According to a third aspect of the present invention there is provided the compound 4-methyl coumarin-7-O-sulphamate.

10 According to a fourth aspect of the present invention there is provided a non-steroidal compound according to the present invention for use as a pharmaceutical.

According to a fifth aspect of the present invention there is provided a non-steroidal compound according to the present invention for inhibiting oestrone sulphatase

15

According to a sixth aspect of the present invention there is provided a pharmaceutical composition comprising a non-steroidal compound according to the present invention; and a pharmaceutically acceptable carrier, excipient or diluent.

20 According to a seventh aspect of the present invention there is provided the use of a non-steroidal compound according to the present invention in the manufacture of a pharmaceutical for inhibiting oestrone sulphatase.

25 According to an eighth aspect of the present invention there is provided a process for preparing a compound according to the present invention, the process comprising sulphating a coumarin.

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According to a ninth aspect of the present invention there is provided a process for preparing a compound according to the present invention, the process comprising sulphamoylating a coumarin.

5 The alkyl group(s) in Formula (A) or Formula (B) can be any suitable linear or branched alkyl group which may be saturated or unsaturated and/or substituted or non-substituted. The alkyl group may even be a cyclic alkyl group. For example, at least two of R_{1-6} are linked to form a further cyclic component.

10 Preferably R_1-R_5 are independently selected from H, alkyl and haloalkyl; preferably wherein R_1-R_5 are independently selected from H, C1-6 alkyl and C1-6 haloalkyl.

Preferably R_1-R_5 are independently selected from H, C1-3 alkyl and C1-3 haloalkyl.

15 Preferably R_1-R_5 are independently selected from H, methyl and halomethyl.

Preferably R_6 is OSO_2NH_2 .

20 Preferably the compound is any one of the compounds shown as Compounds 12-16 in Figure 2.

Preferably the compound is 4-methyl coumarin-7-O-sulphamate.

25 In Formula (A) or Formula (B), two or more of R_1-R_6 may be linked together to form an additional cyclic structure. A typical example of such a compound has the general Formula (C) (see Figure 8), wherein any one of R_3-R_6 is a sulphamate group, and wherein n is an integer. Typically, R_6 is a sulphamate group. A typical sulphamate group is $-\text{OS}(\text{O})(\text{O})-\text{NH}_2$. Preferably n is an integer of from 3 to 10, preferably from

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3 to 7. Optionally, the group $(CH_2)_n$ of Formula (C) can be a substituted alkyl chain.

Typical compounds falling within the general Formula (C) are shown in Figure 9 as
5 compound (D) (where $n=3$), compound (E) (where $n=4$), compound (F) (where $n=5$), compound (G) (where $n=6$), compound (H) (where $n=7$). For these compounds, R_6 is a sulphamate group of the formula $-OS(O)(O)-NH_2$ and each of R_3 - R_5 is H.

- 10 The term "sulphamate" as used herein includes an ester of sulphamic acid, or an ester of an N-substituted derivative of sulphamic acid, or a salt thereof. Thus, the term includes functional groups of the formula: $-O-S(O)(O)-N(R_7)(R_8)$ where R_7 and R_8 are independently selected from H, halo, linear or branched alkyl which may be saturated or unsaturated and/or substituted or non-substituted, aryl, or any other suitable group.
- 15 Preferably, at least one of R_7 and R_8 is H. In a preferred embodiment, each of R_7 and R_8 is H.

The term "mimic" as used herein is used in its normal sense - namely having a different structure but having a similar functional effect.

20

A key advantage of the non-steroidal compound of the present invention is that it is potent *in vivo* and that it has less oestrogenic activity and can therefore be deemed to be a "non-oestrogenic compound". The term "non-oestrogenic compound" as used herein means a compound exhibiting no or substantially no oestrogenic activity.

25

The present invention therefore provides non-steroidal compounds which have a reduced oestrogenic activity. In this regard, the non-steroidal compounds of the present invention act as E1-STS inhibitors.

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Another advantage is that the compounds may not be capable of being metabolised to compounds which display or induce hormonal activity.

5 The compounds of the present invention are also advantageous in that they are orally active.

The compounds of the present invention are further advantageous in that they may have an irreversible effect.

10 The compounds of the present invention are further advantageous in that they may also inhibit DHA-STS.

15 Thus, in a preferred embodiment, the non-steroidal compounds are useful for the treatment of breast cancer. In addition, the non-steroidal compounds are useful for the treatment of non-malignant conditions, such as the prevention of auto-immune diseases, particularly when pharmaceuticals may need to be administered from an early age.

20 A particularly preferred non-steroidal compound according to the present invention is 4-methyl coumarin-7-O-sulphamate. This compound is particularly advantageous in that it acts as a time- and a concentration-dependent inhibitor in a similar manner to EMATE. Another key advantage of this non-steroidal compound is that it is an orally active irreversible compound. In addition, it is not metabolised to compounds with hormonal activity.

25

A highly preferred embodiment of the present invention therefore relates to a pharmaceutical composition comprising 4-methyl coumarin-7-O-sulphamate and a pharmaceutically acceptable carrier, excipient or diluent.

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The present invention therefore relates to non-steroidal compounds which are suitable for use as sulphatase inhibitors.

5 In addition to being potent *in vivo* sulphatase inhibitors, the compounds of the present invention have reduced, or even minimal or no, oestrogenic activity.

Studies have shown that the sulphamates of the present invention inhibit enzyme E1-ETS in intact MCF-7 cells in a dose dependent manner with similar potencies to EMATE.

10

Of the preferred coumarin sulphamates, 4-methylcoumarin-7-*O*-sulphamate together with coumarin-7-*O*-sulphamate appear most active *in vitro*. In this regard, 4-methylcoumarin-7-*O*-sulphamate inhibited E1-STS by 93.3% at 10 μ M with an IC_{50} of 380 nM in intact MCF-7 breast cancer cells. This inactivation was shown to be
15 time- and concentration-dependent inhibitor in a similar way to EMATE. 4-methylcoumarin-7-*O*-sulphamate also inhibited placental microsomal dehydroepiandrosterone sulphatase by 93.6% at 10 μ M. This compound also shows a reduced oestrogenic activity as was seen by the lack of significant increase in the uterine weight in treated ovariectomised rats.

20

The compound also has a potent oral activity.

The non-steroidal compounds of the present invention, in particular the preferred coumarin sulphamates, represent important compounds for the optimisation of non-steroidal sulphatase inhibition. The compounds are also believed to have therapeutic
25 uses other than for the treatment of endocrine-dependent cancers, such as the treatment of autoimmune diseases.

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The present invention will now be described only by way of example with reference to the accompanying drawings in which:-

5 Figure 1 shows the known structures of oestrone (1), oestrone sulphate (2), EMATE (3) and steroid sulphamates (4-5);

Figure 2 shows the structures of 7-hydroxycoumarin (11), 7-(sulphoxy)-4-methylcoumarin (12) and coumarin sulphamates (13-16);

10 Figure 3 shows the sulphation of 7-hydroxy-4-methylcoumarin; pyridine/SO₃-pyridine complex, NaOH in MeOH (*Route a*);

Figure 4 shows the sulphamoylation of 7-hydroxy-4-methylcoumarin; NaH/DMF, H₂NSO₂Cl in toluene (*Route b*);

15 Figure 5 shows the dose-dependent inhibition of oestrone sulphatase in intact MCF-7 breast cancer cells by coumarin-7-*O*-sulphamate (13), 4-methylcoumarin-7-*O*-sulphamate (14), 3,4,8-trimethyl-coumarin-7-*O*-sulphamate (15) and 4-(trifluoromethyl)coumarin-7-*O*-sulphamate (16);

20 Figure 6 shows the time-dependent and the concentration-dependent inactivation of oestrone sulphatase by 4-methyl-coumarin-7-*O*-sulphamate (14);

Figure 7 is a graph;

25

Figure 8 presents Formulae (A), (B) and (C); and

Figure 9 presents Formulae (D), (E), (F), (G) and (H).

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EXAMPLES

The compounds of the present invention may be prepared by a process that comprises a Packman synthesis step. Packman synthesis is known in the art.

5

Sulphamoylation of coumarins

The general procedure for the sulphamoylation of coumarins was as follows. A solution of an appropriate coumarin in anhydrous DMF (ca. 40 ml per g of coumarin) was treated with sodium hydride [60% dispersion; 1 equiv] at 0°C under an atmosphere of N₂. After evolution of hydrogen had ceased, sulphamoyl chloride in toluene [ca. 0.68 M, 1.5 equiv] was added and the reaction mixture was poured into water after warming to room temperature overnight and then the crude product was then quenched. The organic fraction in ethyl acetate (150 ml) was washed exhaustively with brine, dried (MgSO₄), filtered and evaporated. The crude product obtained was purified by flash chromatography followed by recrystallisation to give the corresponding sulphamate. All new compounds were fully characterised by spectroscopic and combustion analysis. The synthesis of 4 methylcoumarin-7-O-sulphamate (14) is shown in Figure 4.

20

Following this general procedure, compounds 13-16 (as shown in Figure 2) - i.e. coumarin-7-O-sulphamate (13), 4-methylcoumarin-7-O-sulphamate (14), 3,4,8-trimethylcoumarin-7-O-sulphamate (15) and 4-(trifluoromethylcoumarin)-7-O-sulphamate (16) - were prepared. More details on the synthesis of these compounds now follow.

25

The synthesis of compound 12 (as shown in Figure 2) is also discussed below:

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Preparation of Coumarin-7-O-sulphamate (13)

Following the above-mentioned general procedure, 7-Hydroxycoumarin (500 mg, 3.082 mmol) gave a crude product (605 mg) which was fractionated on silica (200 g) by gradient elution with chloroform/acetone (8:1, 500 ml; 4:1, 1000 ml and then 2:1, 500 ml). Upon evaporation, the second fraction gave a creamy yellow residue (389 mg, 52.3%) which was recrystallised in ethyl acetate/hexane (1:1) to give (13) as dull white crystals (239 mg).

10 Analytical data were as follows:

M.p. 170.0-171.5°C; R_f = 0.48 (ether), 0.67 (ethyl acetate), 0.19 (chloroform/acetone, 4:1); ν_{\max} (KBr) 3360, 3210, 3060, 1720, 1615, 1370, 1125 cm^{-1} ; δ_H (DMSO- d_6 /CDCl $_3$, ca. 1 : 25) 6.415 (1H, d, $J_{C-4-H, C-3-H}$ = 9.7 Hz, C-3-H), 7.285 (1H, dd, $J_{C-8-H, C-6-H}$ = 2.3 Hz and $J_{C-3-H, C-6-H}$ = 8.5 Hz, C-6-H), 7.379 (1H, d, $J_{C-6-H, C-8-H}$ = 2.2 Hz, C-8-H), 7.508 (2H, br s, D $_2$ O exchanged, -NH $_2$), 7.543 (1H, d, $J_{C-6-H, C-5-H}$ = 8.4 Hz, C-5-H) and 7.760 (1H, d, $J_{C-3-H, C-4-H}$ = 9.7 Hz, C-4-H). MS: m/z (E.I., rel. intensity) 241.0(10), 162.0(97), 134.0(100), 105.0(23). Acc. MS: m/z 241.0068, C $_9$ H $_7$ NO $_5$ S requires 241.0045. Found: C, 44.8; H, 2.89; N, 5.82.

20 C $_9$ H $_7$ NO $_5$ S requires C, 44.81; H, 2.92; N, 5.81%.

Preparation of 4-Methylcoumarin-7-O-sulphamate (14)

Following the above-mentioned general procedure, 7-Hydroxy-4-methylcoumarin (500 mg, 2.753 mmol) gave a crude product (633 mg) which was fractionated on silica (200 g) by gradient elution with chloroform/acetone (8:1, 500 ml; 4:1, 1000 ml, 2:1, 500 ml and then 1:1, 500 ml). Upon evaporation, the second fraction gave a creamy yellow residue (425 mg, 60.5%) which was recrystallised in acetone/chloroform (3:5) to give (14) as colorless rhombic crystals (281 mg).

Analytical data were as follows:

M.p. 165-167°C; R_s = 0.48 (ether), 0.29 (ether/hexane 8 : 1), 0.26 (chloroform/acetone, 4:1); ν_{\max} (KBr) 3320, 3180, 3080, 1700, 1620, 1560, 1380, 1125 cm^{-1} ; δ_{H} (acetone- d_6) 2.507 (3H, s, $-\text{CH}_3$), 6.339 (1H, s, C-3-H), 7.299 (2H, m, C-6-H and C-8-H), 7.390 (2H, br s, D_2O exchanged, $-\text{NH}_2$) and 7.850 (1H, d, $J_{\text{C-6-H, C-5-H}}$ = 9 Hz, C-5-H). MS: m/z (+ve ion FAB in m-NBA, rel. intensity) 542.2(15), 511.1[45, (2M+H)⁺], 461.2(20), 409.1[60, (M+H+NBA)⁺], 393.3[60, (M+H+NBA-16)⁺], 329.2[10, (M+H+NBA-80)⁺], 256.1[100, (M+H)⁺]. MS: m/z (-ve ion FAB in m-NBA, rel. intensity) 421.0(20), 407.1[15, (M-H+NBA)⁻], 335.1(14), 254[100, (M-H)⁻], 175.1[32, (M-H-79)⁻], 121.0(17). Found: C, 47.2; H, 3.56; N, 5.51. $\text{C}_{10}\text{H}_9\text{NO}_5\text{S}$ requires C, 47.06; H, 3.55; N, 5.49%.

Preparation of 3,4,8-Trimethylcoumarin-7-O-sulphamate (15)

15

Following the above-mentioned general procedure, 7-Hydroxy-3,4,8-trimethylcoumarin (1.0 g, 4.896 mmol) gave a crude product (1.33 g) which upon recrystallisation in hot ethyl acetate yielded 238 mg of starting coumarin. The mother liquor was evaporated and the white residue obtained (1.13 g) was fractionated on silica (200 g) with ether. The second fraction was collected, evaporated and the residue obtained (519 mg, 37.4%) was recrystallised in acetone/hexane (1 : 2) to give (15) as pale yellow crystals (312 mg).

Analytical data were as follows:

25

M.p. 197-202°C; R_s = 0.50 (ether), 0.69 (ethyl acetate); ν_{\max} (KBr) 3310, 3040, 1680, 1600 cm^{-1} ; δ_{H} (acetone- d_6) 2.176, 2.383 and 2.458 (9H, three s, 3 x CH_3), 7.374 (1H, d, $J_{\text{C-5-H, C-6-H}}$ = 8.8 Hz, C-6-H), 7.390 (2H, br s, D_2O exchanged, $-\text{NH}_2$) and 7.682 (1H, d, $J_{\text{C-6-H, C-5-H}}$ = 8.8 Hz, C-5-H). MS: m/z (E.I., rel. intensity) 283.1(10), 204.1(45), 176.1(40), 161.1(22), 69.1(56), 57.1(40), 43.1(100). Acc.

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MS: m/z 283.0497, $C_{12}H_{13}NO_5S$ requires 283.0514. Found: C, 50.86; H, 4.63; N, 4.97. $C_{12}H_{13}NO_5S$ requires C, 50.88; H, 4.63; N, 4.94%.

Preparation of 4-(Trifluoromethyl)coumarin-7-O-sulphamate (16)

5

Following the above-mentioned general procedure, 7-Hydroxy-4-(trifluoromethyl)-coumarin (0.90 g, 3.911 mmol) gave a crude product (1.20 g) which was fractionated on silica (200 g) with ether/chloroform (1:4). The residue (392 mg) from the third fraction was further purified by fractionating on silica (100 g) with ether. The first 10 fraction then collected gave a residue (295 mg, 24.4%) which upon recrystallised in ethyl acetate/hexane (1 : 3) gave (16) as white needle-shaped crystals (160 mg).

Analytical data were as follows:

15 M.p. 165-168°C; R_f s = 0.67 (ether), 0.24 (ether/chloroform, 1 : 4); ν_{max} (KBr) 3360, 3240, 3100, 1720, 1620, 1380, 1160 cm^{-1} ; δ_H (acetone- d_6) 6.995 (1H, s, C-3-H), 7.461 (1H, dd, $J_{C-8-H, C-6-H}$ = 2.8 Hz and $J_{C-5-H, C-6-H}$ = 8.1 Hz, C-6-H), 7.478 (1H, s, C-8-H), 7.53 (2H, br s, D_2O exchanged, $-NH_2$) and 7.89 (1H, m, C-5-H). 1H -NMR spectrum of (16) in $DMSO-d_6/CDCl_3$ (ca. 1 : 15) showed partial decomposition 20 to the starting coumarin. MS: m/z (E.I., rel. intensity) 309.0(2.6), 230.0(77), 202.0(100), 183.5(5), 173.0(10), 69.0(33). Acc. MS: m/z 308.9874, $C_{10}H_6F_3NO_5S$ requires 308.9919. Found: C, 38.8; H, 1.85; N, 4.53. $C_{10}H_6F_3NO_5S$ requires C, 38.84; H, 1.96; N, 4.53%.

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Preparation of 7-(Sulphoxy)-4-Methylcoumarin, sodium salt (12)

To a solution of 7-hydroxy-4-methylcoumarin (1.0 g, 5.676 mmol) in dried pyridine (20 ml) under an atmosphere of N₂ [Figure 3] was added sulphur trioxide-pyridine complex (1.8 g, 11.35 mmol, 2 equiv.) and the reaction mixture was stirred overnight. After removal of pyridine, methanol (20 ml) was added to the creamy syrup obtained and the resulting light yellow solution was basified (pH ~ 8) by dropwise addition of sodium hydroxide in methanol (1 M, ca. 18 ml). The bright yellow suspension formed was filtered and the precipitate washed with more methanol. The filtrate was then concentrated to 30-40 ml and ether (total 120 ml) was added in portions until precipitation completed. The light beige precipitate was collected (711 mg) and 582 mg of which was recrystallised in methanol/ether (1 : 1) to give (12) as light creamy yellow crystals (335 mg).

15 Analytical data were as follows:

M.p. 172-175°C (dec.); R_f = 0.51 (methanol/ethyl acetate, 1 : 3), 0.67 (methanol/ether, 1 : 3); ν_{max} (KBr) 3500 (br), 3080, 1680, 1610, 1560, 1300, 1260, 1050 cm⁻¹; δ_H (DMSO-d₆) 2.407 (3H, s, -CH₃), 6.269 (1H, s, C-3-H), 7.20 (2H, m, C-6-H and C-8-H), and 7.695 (1H, d, J_{C-6-H, C-5-H} = 8.8 Hz, C-5-H). MS: m/z (+ve ion FAB in m-NBA, rel. intensity) 176(100, NBA+Na⁺). MS: m/z (-ve ion FAB in m-NBA, rel. intensity) 175.1 (14, M-Na⁺-SO₃), 255.0 (100, M-Na⁺), 408.0 (8, M-Na⁺+NBA), 431.0 (15, M+153), 444.0(20), 533.0(15), 230.0(77), 202.0(100), 183.5(5), 173.0(10), 69.0(33). Acc. MS: m/z (-ve ion FAB in glycerol, rel. intensity) 254.9982(25), C₁₀H₇O₆S requires 254.9963. Found: C, 40.3; H, 2.92. C₁₀H₇O₆NaS·H₂O requires C, 40.55; H, 3.06%. HPLC [Spherisorb ODS5, 25x4.6 mm; Mobile phase: MeOH/H₂O (70 : 30), Flow rate: 1 ml/min; λ_{max}: 316 nm]: t_R = 1.5 min, c.f. 7-hydroxy-4-methylcoumarin, 3.6 min.

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Other data were as follows:

Compound 12 is stable in bases such as sodium hydroxide in methanol but not in acidic conditions. In addition, incomplete basification of the reaction mixture with sodium hydroxide in methanol (< 3 equivalents) leads to decomposition of (12). Two equivalents of sodium hydroxide are required for consuming excess sulphur trioxide-pyridine complex to yield the neutral sodium sulphate. Insufficient amount of sodium hydroxide will therefore lead to the formation of sodium hydrogen sulphate which is acidic. Compound 12 appears labile to high temperature as one experiment has shown complete decomposition to 7-hydroxy-4-methylcoumarin after heating (12) as solid at 90°C for 4 h.

In vitro tests

The above-mentioned coumarin sulphamates were tested for their ability to inhibit E1-STS activity using intact MCF-7 breast cancer cells or placental microsomes (100,000g fraction) essentially as previously described.

To examine whether compound (12) could act as a substrate for E1-STS, 100 µg of the compound was incubated for 1 hour with placental microsomes in the absence or presence of EMATE (10 µM). The unconjugated coumarin formed at the end of the incubation was extracted with diethyl ether. After evaporation of solvent, the residue was examined by TLC using ethyl acetate/methanol (80:20) as eluent, in which the coumarin sulphate (12) and 7-hydroxy-4-methylcoumarin had R_f values of 0.79 and 0.95 respectively. Only unconjugated 7-hydroxy-4-methylcoumarin was detected after incubation of compound (12) with placental microsomes. The inclusion of EMATE in the reaction mixture reduced the hydrolysis of compound (12) by E1-STS, indicating that the coumarin sulphate is indeed a substrate for the sulphotase.

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The dose-dependent inhibition of oestrone sulphatase in intact MCF-7 breast cancer cells by coumarin-7-*O*-sulphamate (13), 4-methylcoumarin-7-*O*-sulphamate (14), 3,4,8-trimethyl-coumarin-7-*O*-sulphamate (15) and 4-(trifluoromethyl)coumarin-7-*O*-sulphamate (16) can be seen from Figure 5. Assays were performed essentially as previously described. (7, 8) Monolayers of intact MCF-7 cells in 25 cm² flasks were incubated for 20 h at 37 °C with [³H]oestrone sulphate (2 nM) and coumarin sulphamates at 0.1-10 μM. Oestrone sulphatase activity was determined by measuring the total amount of ³H-labeled oestrone and oestradiol formed. Sulphatase activity in untreated cells was 100-200 fmol/20 h/10⁶ cells. Each point represents the mean ± s.d. of triplicate measurements.

The free parent coumarins of all coumarin sulphamates prepared showed little or no E1-STS inhibitory activity when tested up to 10 μM. However, in contrast, all four coumarin sulphamates (compounds 13-16) inhibited oestrone sulphatase inhibitory activity in a dose-dependent manner (Figure 5) and the inhibition at 10 μM ranged from 71.5% for compound 16 to 93.3% for compound 14. The IC₅₀ for inhibition of E1-STS by compound 14, the most effective inhibitor, measured using intact MCF-7 cells was 380 nM.

The time- and concentration-dependent inactivation of oestrone sulphatase by 4-methyl-coumarin-7-*O*-sulphamate (14) can be seen from Figure 6. Placental microsomes (200 μg) were preincubated with (14) (control, ●; 0.5 μM, Δ and 10 μM, ★) for 0-30 min at 37°C followed by incubation with dextran-charcoal for 10 min at 4°C. Dextran-charcoal was sedimented by centrifugation and portions of the supernatants were then incubated with [³H]oestrone sulphate (20 μM) for 1 h at 37°C to assess remaining sulphatase activity. Duplicate experiments were run at each concentration, but assays for residual activity were taken at different times in each experiment.

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As with EMATE, compound 14 inhibited E1-STS activity in a time- and concentration-dependent manner in a biphasic fashion (Figure 6), indicating a similar mechanism of action (potential chemical modification of two active site residues). At 10 μ M, compound 14 reduced the original E1-STS activity by 95% after preincubating the enzyme with the inhibitor for 20 min.

Additional experiments revealed that compound 14 inhibited placental microsomal DHA-STS activity by 93.6% at the same concentration.

10 In Vivo Tests

In order to examine if compound 14 possessed oestrogenic activity and also to test its ability to inhibit E1-STS *in vivo*, it was administered to rats (1mg/kg subcutaneously, in propylene glycol for 5 days) 14 days after ovariectomy had been performed.

15

Administration of compound 14 did not result in any significant increase in the uterine weight in these rats (data not shown), showing that compound 14 showed reduced oestrogenic agonist properties. The E1-STS activity in the uteri obtained from these animals was inhibited by 89.4% compared with the activity in untreated animals.

20

Preliminary data also demonstrate potent oral activity in rats for compound 14, similar to that observed for EMATE.

In addition to these *in vivo* results, another series of rats (each weighing approximately 200g) received 4-methyl coumarin-7-0-sulphamate (compound 14) orally in propylene glycol either as a single dose (SD) or daily for seven days (Multiple Dose, MD).

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Inhibition of sulphatase activity was assessed in white blood cells (wbcs) that were collected after a SD or MD. Sulphatase activity was assayed using labelled oestrone sulphate as the substrate and measuring the release of oestrone.

- 5 The results are shown in Figure 7 and in the Table below:

	Dose mg/kg	% Inhibition	
		SD	MD
10	0.1	72	65
	1.0	85	85
	10.0	96	89

Similar results were found with liver cells.

- 15 Compound 14 therefore demonstrates potent oral activity.

Other modifications of the present invention will be apparent to those skilled in the art.

SUBSTITUTE SHEET (RULE 26)

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CLAIMS

1. A non-steroidal sulphamate compound suitable for use as an inhibitor of oestrone sulphatase, wherein the compound has a polycyclic ring structure comprising
5 at least a first ring and a second ring; wherein the first ring and the second ring mimic the A and B rings of oestrone; and wherein the polycyclic ring structure is not tetrahydro-naphthol.
2. A non-steroidal sulphamate compound according to claim 1 wherein either the
10 first ring or the second ring comprises an α,β -unsaturated lactone group.
3. A non-steroidal compound according to claim 1 or claim 2, wherein the first ring is a phenolic ring.
- 15 4. A non-steroidal compound according to any one of the preceding claims wherein the compound has a bicyclic ring structure.
5. A non-steroidal sulphamate compound according to any one of the preceding claims wherein the compound has the general Formula (A), wherein R_1-R_6 are
20 independently selected from H, halo, hydroxy, sulphamate, alkyl and substituted variants or salts thereof; but wherein at least one of R_1-R_6 is a sulphamate group; and wherein X is any one of O, S, NH, a substituted N, CH_2 , or a substituted C.
6. A non-steroidal sulphamate compound according to claim 5 wherein X is O.
25
7. A non-steroidal sulphamate compound according to any one of the preceding claims wherein the compound is not hydrolysable by an enzyme having steroid sulphatase (E.C. 3.1.6.2) activity.

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8. A non-steroidal compound wherein the compound has the general Formula (B) wherein R_1-R_5 are independently selected from H, halo, hydroxy, sulphamate, alkyl and substituted variants or salts thereof; but wherein at least one of R_1-R_5 is a sulphamate group.

5

9. A non-steroidal compound according to claim 8, wherein R_1-R_5 are independently selected from H, alkyl and haloalkyl; preferably wherein R_1-R_5 are independently selected from H, C1-6 alkyl and C1-6 haloalkyl.

10. A non-steroidal compound according to claim 9, wherein R_1-R_5 are independently selected from H, C1-3 alkyl and C1-3 haloalkyl.

11. A non-steroidal compound according to claim 10, wherein R_1-R_5 are independently selected from H, methyl and halomethyl.

15

12. A non-steroidal compound according to any one of claims 8 to 11 wherein R_6 is OSO_2NH_2 .

13. A non-steroidal compound according to any one of the preceding claims wherein the compound is any one of the compounds shown as Compounds 12-16 in Figure 2.

20

14. A non-steroidal compound according to any one of the preceding claims, wherein the compound is 4-methyl coumarin-7-O-sulphamate.

25

15. A non-steroidal compound according to any one of the preceding claims for use as a pharmaceutical.

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16. A non-steroidal compound according to any one of claims 1 to 15 for inhibiting oestrone sulphatase
17. A pharmaceutical composition comprising a non-steroidal compound according to any one of claims 1 to 14; and a pharmaceutically acceptable carrier, excipient or diluent.
18. Use of a non-steroidal compound according to any one of claims 1 to 14 in the manufacture of a pharmaceutical for inhibiting oestrone sulphatase.
19. A process for preparing a compound according to any one of the preceding claims, the process comprising sulphating a coumarin.
20. A process for preparing a compound according to any one of the preceding claims, the process comprising sulphamoylating a coumarin.
21. A non-steroidal compound substantially as described herein.
22. A process of preparing a non-steroidal compound substantially as described herein.

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- \bar{X}
- (1) -OH
- (2) -OSO₃⁻
- (3) -OSO₂NH₂
- (4) -NHISO₂NH₂
- (5) -SSO₂NH₂

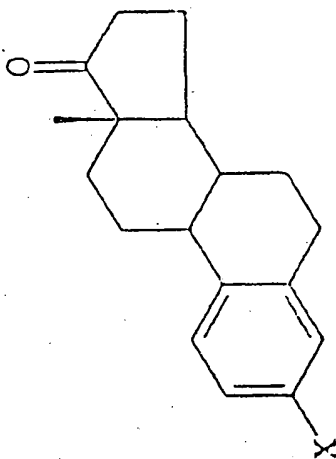


Fig. 1

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X	R ₁	R ₂	R ₃
(11) -OH	H	H	H
(12) -OSO ₃ ⁻	H	CH ₃	H
(13) -OSO ₂ NH ₂	H	H	H
(14) -OSO ₂ NH ₂	H	CH ₃	H
(15) -OSO ₂ NH ₂	CH ₃	CH ₃	CH ₃
(16) -OSO ₂ NH ₂	H	CF ₃	H

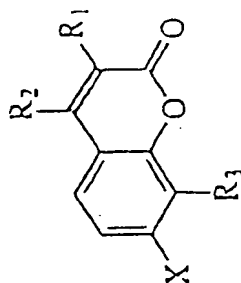


Fig 2

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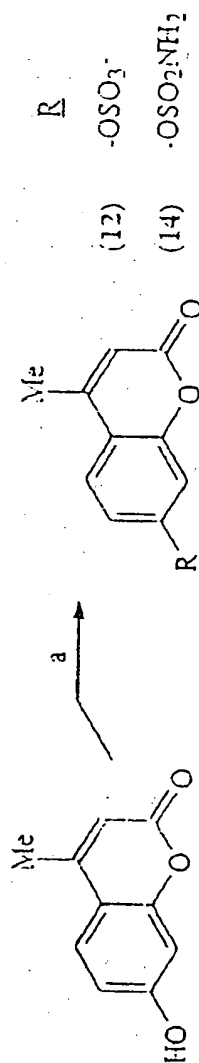


Fig 3

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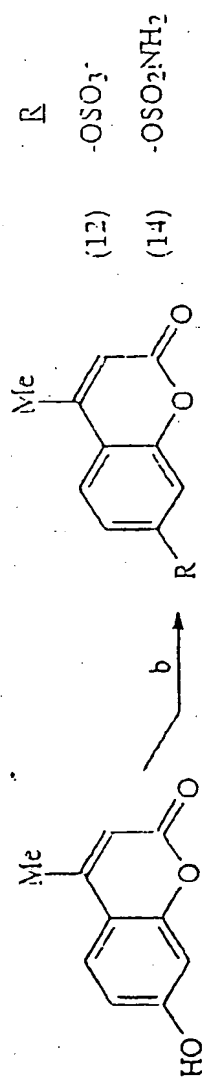


Fig 4

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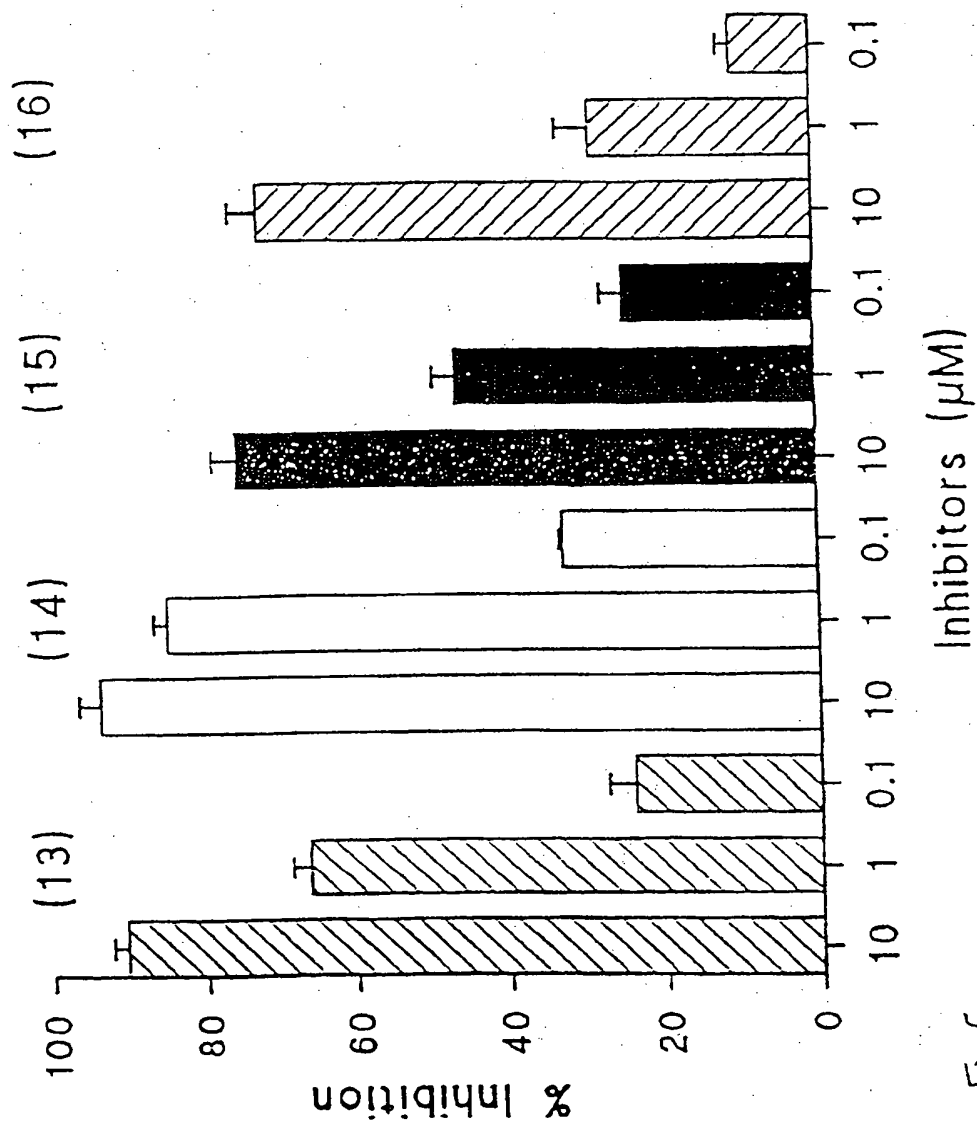


Fig 5

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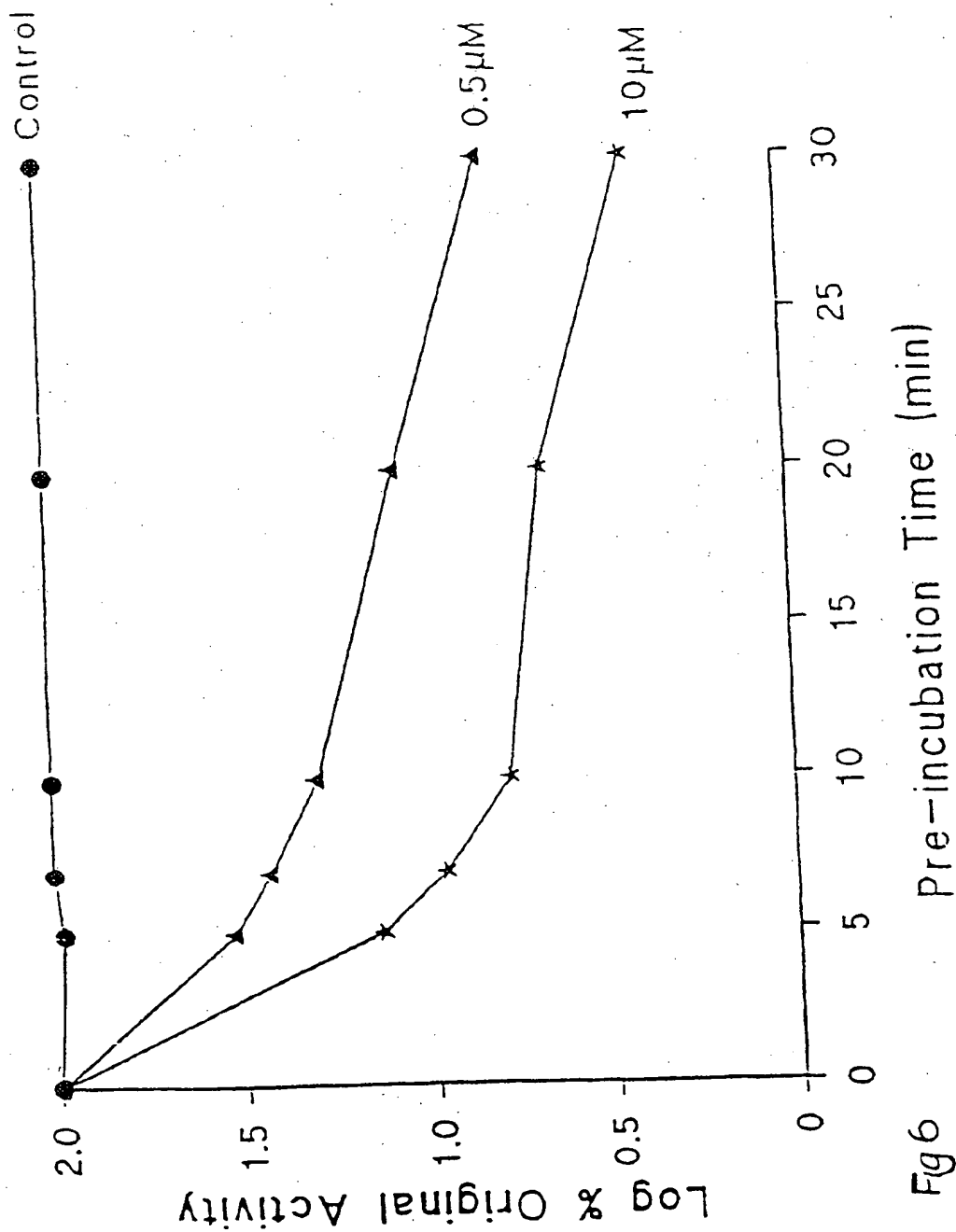
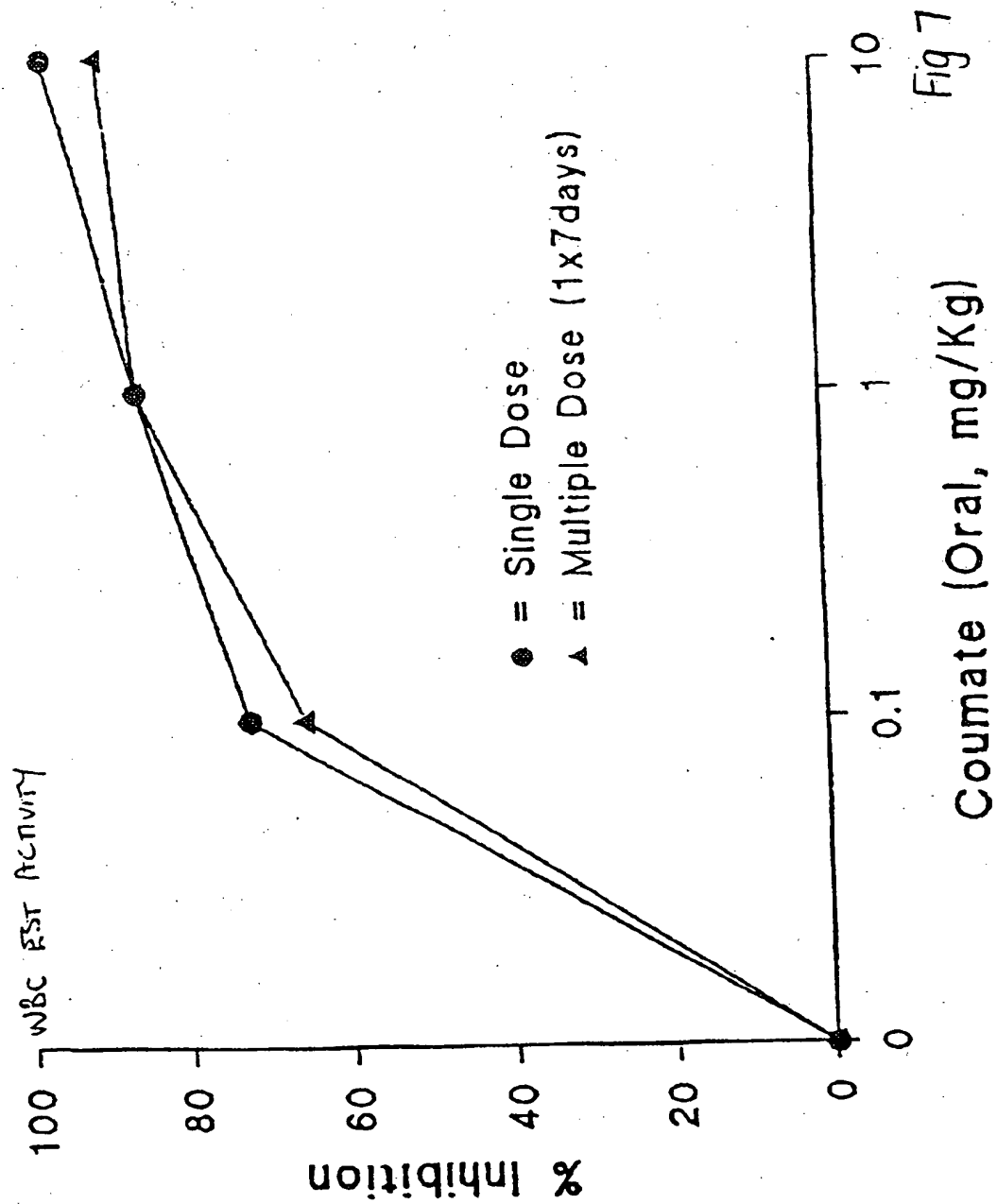


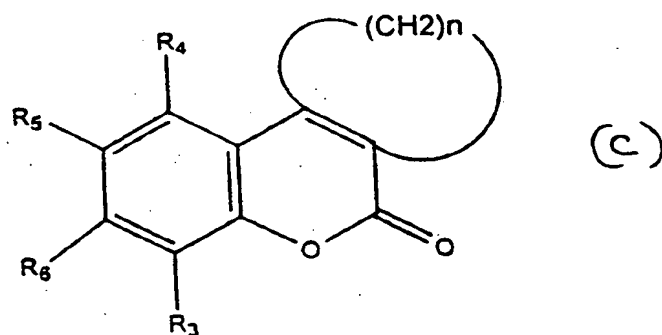
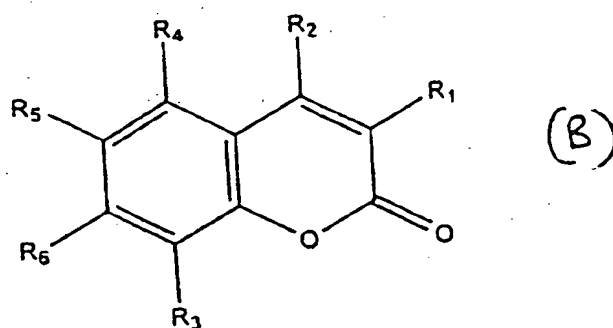
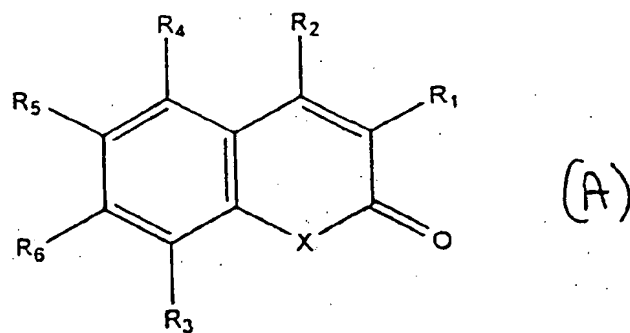
Fig 6

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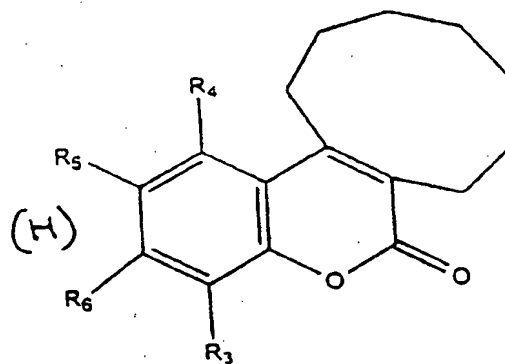
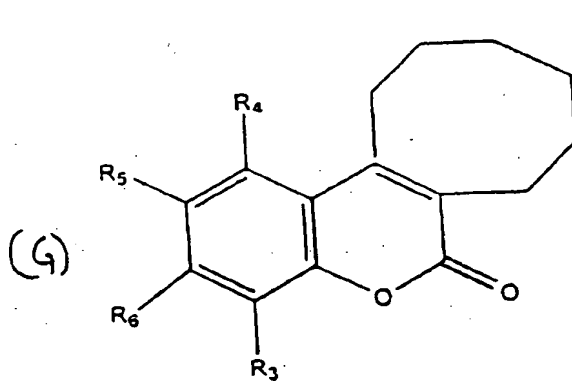
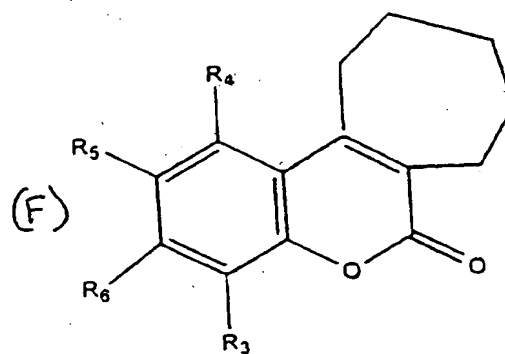
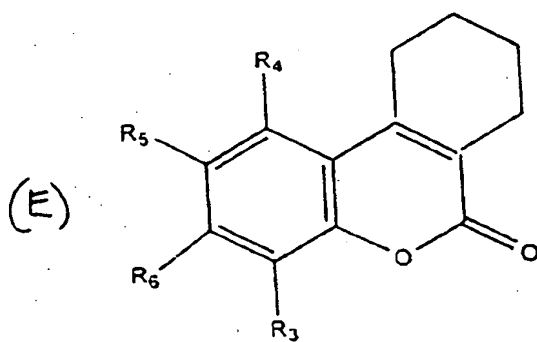
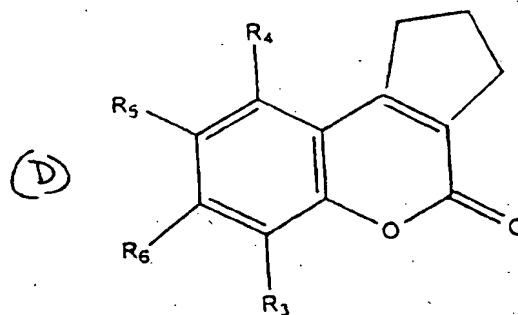


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Fig 8



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INTERNATIONAL SEARCH REPORT

In tional Application No
PCT/GB 97/00444

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07D311/16 A61K31/37

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHEMICAL ABSTRACTS, vol. 62, no. 7, 29 March 1965 Columbus, Ohio, US; abstract no. 7670a, CORRAL C. & MUNICIO A.M.: "Synthesis and anticholinesterase activity of a series of aryl N,N-dimethylsulfamates" column 2; XP002030685 see abstract & ANALES REAL SOC. ESPAN. FIS. QUIM. (MADRID), SER. B, vol. 60, no. 4, 1964, pages 341-344, --- -/--	1,2,4-6, 8,15

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

- * "A" document defining the general state of the art which is not considered to be of particular relevance
- * "E" earlier document but published on or after the international filing date
- * "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- * "O" document referring to an oral disclosure, use, exhibition or other means
- * "P" document published prior to the international filing date but later than the priority date claimed

- * "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- * "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- * "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- * "A" document member of the same patent family

Date of the actual completion of the international search

9 May 1997

Date of mailing of the international search report

23.07.97

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 97/00444

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 111 746 A (BASF AKTIENGESELLSCHAFT) 27 June 1984 see claims 1,3,4; example 43 ---	1-6, 8-11,15, 17,19,20
X	EP 0 403 185 A (A.H. ROBINS COMPANY, INCORPORATED) 19 December 1990 see claims 2,3,6; example 93 ---	1-6, 8-15,17, 19,20
P,X	JOURNAL OF MEDICINAL CHEMISTRY, vol. 39, no. 7, 29 March 1996, pages 1349-1351, XP000652210 WOOD L.W.L. ET AL.: "Active site directed inhibition of estrone sulfatase by nonsteroidal coumarin sulfamates" see the whole document ---	1-20
A	WO 93 05064 A (IMPERIAL COLLEGE OF SCIENCE, TECHNOLOGY AND MEDICINE) 18 March 1993 cited in the application see claims 1,2,6-8,11,12 ---	1,3,7, 15-18
A	JOURNAL OF MEDICINAL CHEMISTRY, vol. 37, no. 2, 21 January 1994, pages 219-221, XP000652211 HOWARTH N.M. ET AL.: "Estrone sulfamates: Potent inhibitors of estrone sulfatase with therapeutic potential" -----	1-20

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

- 1- Claims : 1 (partially), 2, 3-5 (all partially), 6, 7 (partially), 8-14, 15-18 (all partially), 19 and 20.
- 2- Claims : 1 (partially), 3-5 (all partially), 7 (partially) and 15-18 (all partially).
- 3- Claims : 1 (partially), 3 (partially), 7 (partially) and 15-18 (all partially).
- 4- Claims : 21 and 22

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Claims 1 (partially), 2, 3-5 (all partially), 6, 7 (partially), 8-14, 15-18 (all partially), 19 and 20.

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 97/00444

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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